II. REMARKS

A. Status of the Claims

Claims 1-35 and 37-45 were pending in the case at the time of the Office Action. Claims 1, 14, and 31 have been amended in the Amendment set forth herein. Claims 2-6, 15-16, 19, 22-23, 32-34, and 36-38 have been canceled without prejudice or disclaimer. New claims 46 and 47 have been added. Therefore, claims 1, 7-14, 17-18, 20-21, 24-31, 35, and 39-47 are currently under consideration.

Applicant reserves the right to prosecute any subject matter canceled from the originally filed listing of claims in a continuation or divisional application.

Support for the amendments to the claims can be found generally throughout the specification, such as in the originally filed claims, and page 4, lines 30-35 ("graft comprising muscle cells"). Support for the new claims can be found generally throughout the specification, such as in the originally filed claims, page 9, line 20-23, and page 10, lines 1-4 ("hematopoietic stem cells")

B. The Rejections Under 35 U.S.C. §102(b) Are Overcome

1. The Rejections Based on Kocher *et al.*

Claims 1, 5, 6, 10-14, 17, 18, 20, 22, 23, 27-31, 35, 37, 38, 42-44, and 45 are rejected under 35 U.S.C. §102(b) as being anticipated by Kocher *et al.* (Nature Medicine, April 2001, Vol. 7, No. 4, pp. 430-436; "Kocher"). Applicant respectfully traverses this rejection.

Applicant notes that independent claim 1 has been amended to recite the limitations of claims 3-4, claim 14 has been amended to recite the limitations of claims 16 and 19, and claim 31 has been amended to recite the limitations of claims 33-34. Because claims 3-4, 16, 19, and 33-34 were not subject to this rejection, the rejection of each of the claims at issue in this

rejection is moot. Further, new claims 46 and 47 would not be anticipated by Kocher because these claims depend from claim 1.

By amending the claims in this manner, Applicant in no way concedes that the claims as originally written were anticipated by Kocher. Applicant reserves the right to prosecute any subject matter canceled from the claims as originally filed in a continuation or divisional application.

2. The Rejections Based on Kalka *et al.*

Claims 1, 9-14, 17, 18, 21, 26-31, and 41-45 are rejected under 35 U.S.C. §102(b) as being anticipated by Kalka *et al.* (PNAS, March 28, 2000, Vol. 97, No. 7, pp. 3422-3427; "Kalka"). Applicant respectfully traverses.

Applicant notes that independent claim 1 has been amended to recite the limitations of claims 3-4, claim 14 has been amended to recite the limitations of claims 16 and 19, and claim 31 has been amended to recite the limitations of claims 33-34. Because claims 3-4, 16, 19, and 33-34 were not subject to this rejection, the rejection of each of the claims at issue in this rejection is moot. By amending the claims in this manner, Applicant in no way concedes that the claims as originally written were anticipated by Kalka. Further, new claims 46 and 47 would not be anticipated by Kalka because these claims depend from claim 1.

Applicant reserves the right to prosecute any subject matter canceled from the claims as originally filed in a continuation or divisional application.

C. The Rejections Under 35 U.S.C. §103(a) Are Overcome

1. Rejections Based on Kocher and Kalka In View of Gillis

Claims 1-35 and 36-45 are rejected under 35 U.S.C. §103(a) as being unpatentable over Kocher and Kalka in view of U.S. Patent 5,199,942 ("Gillis"). Applicant respectfully traverses.

In order to establish a *prima facie* case of obviousness, three basic criteria must be met: (1) the prior art reference (or references when combined) must teach or suggest all the claim limitations; (2) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (3) there must be a reasonable expectation of success. *Manual of Patent Examining Procedure* § 2142. See also *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q. 2d 1438 (Fed Cir. 1991). All three elements must be shown to establish a *prima facie* case of obviousness. Thus, if one element is missing, a *prima facie* case of obviousness does not exist.

a) Kocher, Kalka, and Gillis Fail to Teach or Suggest Each Limitation of the Claimed Invention

There is no *prima facie* case of obviousness because Kocher and Kalka in view of Gillis does not teach or suggest each limitation of the claimed invention. In particular, none of the cited references appear to teach the limitation of "a donor who is HLA-matched to the subject and genetically non-identical to the subject." As to Kocher and Kalka, the Examiner did not include claims that recite a "donor who is HLA-matched to the subject" (claims 3, 16, and 33) in the rejections under 35 U.S.C. §102(b), thus appearing to admit that these references do not teach or suggest this limitation. Furthermore, Gillis pertains to autologous hematopoietic cell transplantation, and not allogeneic transplantation. See entire document, including abstract and field of the invention (col. 1, lines 12-25). Applicants invite the Examiner to identify any such teaching or suggestion in the cited combination of references.

Further, none of the cited references teach or suggest "producing a graft comprising muscle cells." The Examiner cites to Fig. 2e of Kocher as allegedly teaching this limitation. However, this figure legend, which recites "endothelial cells lining numerous capillaries (arrows)

within myocardial infarct bed," does not teach or suggest the development of ***muscle cells***. Further, the sections of Kalka cited by the Examiner which allegedly teach this limitation do not pertain to the development of muscle cells but address neovascularization. Gillis is not cited as teaching or suggesting this limitation, nor does Applicant identify any such information in Gillis.

Therefore, because Kocher, Kalka and Gillis do not teach or suggest each limitation of the claimed invention, there can be no *prima facie* case of obviousness.

b) Kocher and Kalka in View of Gillis Fails to Provide Any Suggestion or Motivation to One of Ordinary Skill in the Art to Practice the Claimed Invention

There is further no *prima facie* case of obviousness because Kocher and Kalka in view of Gillis fails to provide any suggestion or motivation to one of ordinary skill in the art to practice the claimed invention. As discussed above, none of the cited references pertain to production of a graft comprising muscle cells. Further, the claims as presently set forth pertain to allogeneic transplantation from a donor into a human subject. Neither Kocher nor Kalka teach or suggest this limitation. Further, Gillis provides no motivation to provide for this missing information because it pertains to autologous transplantation, and not allogeneic transplantation.

c) The New Claims are Non-Obvious

As discussed above, new claims 46 and 47 have been added. These claims depend from claim 1. For the reasons discussed above pertaining to the remaining claims at issue in this rejection, there is no *prima facie* case of obviousness.

d) Conclusion

In view of the above, there is no *prima facie* case of obviousness as to each of the cited claims. Therefore, it is respectfully requested that this rejection should be withdrawn.

2. Rejections Based on Pittenger and Fernandez In View of Orlic

Claims 1-35 and 37-45 are rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent 6,387,369 ("Pittenger") and U.S. Patent 6,261,549 ("Fernandez") taken with Orlic *et al.* (Nature, 2001, Vol. 410, pp. 701-705; "Orlic - 1") or Orlic *et al.* (Blood 2001, Vol. 98, No. 11, part 1, page 810a; "Orlic-2"). Applicant respectfully traverses.

a) Pittenger and Fernandez in View of Orlic-1 and Orlic-2 Fail to Teach or Suggest Each Limitation of the Claimed Invention

There is no *prima facie* case of obviousness because Pittenger and Fernandez in view of Orlic does not teach or suggest each limitation of the claimed invention. In particular, none of the cited references teach the limitation of "a donor who is HLA-matched to the subject and genetically non-identical to the subject," a limitation which is present in each of the pending claims. Fernandez appears silent as to the HLA status of the donor. Pittenger discloses that the stem cells used in its methods are "preferably autologous" rather than genetically non-identical. See col. 1, lines 45-48. Neither of the Orlic references appear to address HLA-matching of the subjects. Applicants invite the Examiner to identify any such teaching or suggestion in the cited combination of references. Furthermore, none of the cited references teach or suggest any of the mobilization factors set forth in any of claims 1, 14, and 31.

Regarding new claims 46 and 47, there is no prima facie case of obviousness for the reasons discussed above, as these claims depend from claim 1.

There is additionally no *prima facie* case of obviousness as to claim 46 because Pittenger and Fernandez in view of Orlic-1 and Orlic-2 fails to provide any suggestion or motivation to one of ordinary skill in the art to provide for use of hematopoietic cells. Pittenger and Fernandez do not pertain to methods involving hematopoietic stem cells, but pertian to methods involving mesenchymal cells. *See, e.g.*, Pittenger, col. 1, lines 42-45 and abstract of Fernandez. It is

known in the art that mesenchymal stem cells and hematopoietic stem cells are distinct types of adult stem cells. See, *e.g.*, page 929 of Strauer and Kornowski, Circulation 107:929-934, 2003(Exhibit 1) and pages 1-2 of Exhibit 2 ("Types of Stem Cells," Wilkipedia). In addition, neither of the Orlic references appear to provide any teaching or suggestion to one of ordinary skill in the art to use hematopoietic cells in the techniques taught by Pittenger. Thus, in the absence of any teaching or suggestion to combine reference teachings, there can be no *prima facie* case of obviousness.

Therefore, because Kocher, Kalka and Gillis do not teach or suggest each limitation of the claimed invention, there can be no *prima facie* case of obviousness.

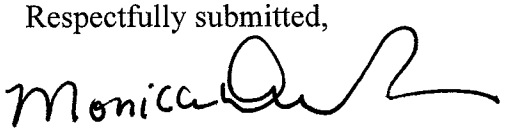
b) Conclusion

In view of the above, there is no *prima facie* case of obviousness as to each of the cited claims. Therefore, it is respectfully requested that this rejection should be withdrawn.

D. Conclusion

In view of the foregoing, each of the pending claims is in condition for allowance, and a Notice of Allowance is earnestly solicited. The Examiner is invited to contact the undersigned attorney at 512.536.5639 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



Monica A. De La Paz
Reg. No. 54,662
Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
512.474.5201 (telephone)
512.536.4598 (fax)

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EXHIBIT 1

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Stem Cell Therapy in Perspective

Bodo E. Strauer and Ran Kornowski

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Stem Cell Therapy in Perspective

Bodo E. Strauer, MD; Ran Kornowski, MD

The concept of regenerative medicine using the body's own stem cells and growth factors to repair tissues may become a reality as new basic science works and initial clinical experiences have "teamed-up" in an effort to develop alternative therapeutic strategies to treat the diseased myocardium. In particular, revealing the signals that mediate cellular growth and differentiation may provide novel tools designed for myocardial regeneration in patients sustaining ischemic cardiomyopathy syndromes. We attempt herein to provide a critical overview of recent developments of myocardial cell transplantation strategies.

Stem Cells

Stem cells are a population of immature tissue precursor cells capable of self-renewal and provision of de novo and/or replacement cells for many tissues. Embryonic stem cells can be obtained from the inner cell mass of the embryonal blastocyst. Although it was recently shown that human embryonic stem cells can differentiate into cardiomyocytes,[1] because of the immunogenicity and rejection, as well as ethical considerations, these cells may be restricted to experimental in vitro studies and their therapeutical potential remains to be determined. Also, these cells may act as an unanticipated arrhythmogenic source after intramyocardial transplantation.[2] Clinical application of these cells is most likely years ahead (Table).

In contrast, adult human stem cells (hematopoietic, mesenchymal) are found in mature tissues, eg, the bone marrow. Plasticity of adult stem cells can probably generate lineages of cells different from their original organ of origin. Thus, these cells can be used for organ regeneration and for cellular repair in various species, as well as in humans.

Ethical problems for adult autologous stem cells do not exist, and although much experimental work remains to be done, their clinical relevance and therapeutic benefit in heart disease have recently been shown for the first time.[3]

Except for hematopoietic and mesenchymal stem cells, many other bone marrow-related cell types may participate in organ repair of infarction models; bone marrow hemangioblasts take part in neovascularization, mesodermal progenitor cells are contained within the mononuclear bone marrow cell fraction that differentiates to endothelial cells, and endothelial progenitor cells can transdifferentiate into cardiomyocytes. Primitive bone marrow cells mobilized by stem cell factor and granulocyte-colony stimulating factor are capable of homing to infarct regions, replicating, differentiating, and promoting myocardial repair.[4] Ultimately, a variety of different cell types from the mononuclear bone marrow cell fraction contribute to the regeneration of necrotic myocardium and damaged vessels. In this regard, therapeutic use of mononuclear cell populations of bone marrow may be more useful and promising than single isolated cell fractions alone. The effect manifested by more heterogenous bone marrow cell populations that contain very small numbers of stem cells may also suggest the importance of an entire array of bone marrow-derived growth factors and cytokines that may also regulate cellular growth and regeneration via cellular secretion mechanisms.

Stem Cells and Angiogenesis

The complex cellular and molecular mechanisms by which endothelial and smooth-muscle cells interact with each other to form blood vessels are now better understood.[5] Endothelial cells alone can initiate the formation and sprouting of

From the Department of Medicine, Division of Cardiology, Heinrich-Heine Universitaet Duesseldorf, Duessledorf, Germany (B.E.S.); and Cardiology Department, Rabin Medical Center, Petach-Tikva, Israel (R.K.).

Correspondence to Prof Dr med B.E. Strauer, MD, Department of Medicine, Division of Cardiology, Heinrich-Heine Universitaet Duesseldorf, Moorenstr. 5, 40225 Duessledorf, Germany. E-mail strauer@med.uni-duesseldorf.de

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Advantages and Disadvantages of Embryonic Versus Adult Stem Cells

	Embryonic Stem Cells	Adult Stem Cells
Advantages	Highly expandable	Easily obtainable
	Pluripotent	No ethical objections
		Different expansion ability
		Uni-, bi-, multi- or pluripotent
		Highly compatible
		Autologous transplantation, no immune-suppressive therapy necessary
		Clinical application already realized
Disadvantages	Ethical objections	Lack of specific identification markers
	Difficult to isolate	
	Risk of rejection	
	Immune-suppressive therapy required	
	Arrhythmogenic potential	
	High risk of teratocarcinomas	
	Clinical application not feasible for 10 to 20 years	
	Lack of specific identification markers	

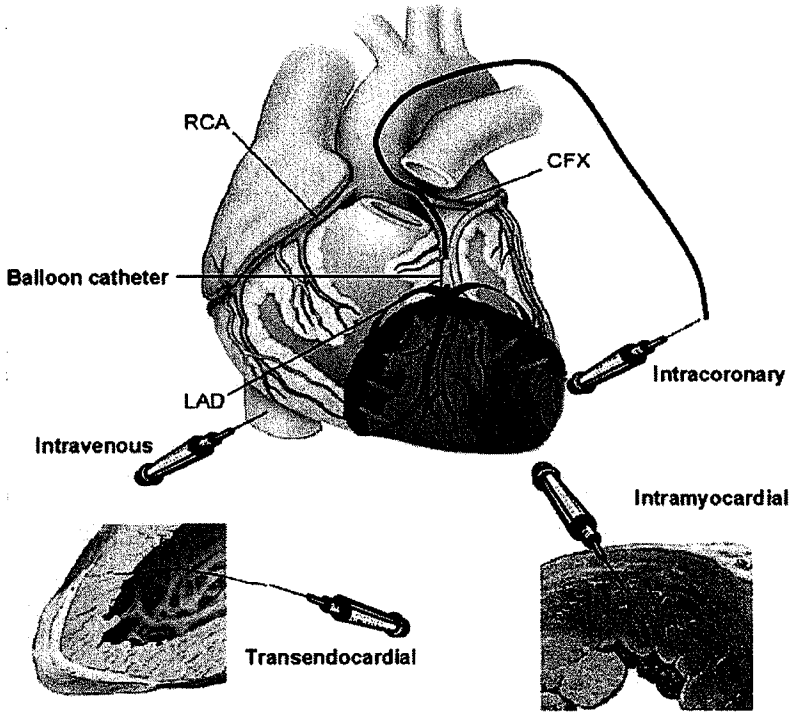
endothelium-lined channels, namely angiogenesis, in response to a physiological or pathological stimulus. Peri-endothelial cells are required for vascular maturation. Recruitment of smooth muscle cells provides these vessels with essential viscoelastic and vasomotor properties and enables accommodating the changing needs in tissue perfusion. This later stage is called arteriogenesis and has a major role in collateral growth.[6] Endothelial progenitor cells could be isolated from peripheral blood and/or bone marrow and showed incorporation into sites of physiological and pathological neovascularization in vivo after either systemic injection or using direct intramyocardial transplantation.[7] In contrast to differentiated endothelial cells, transplantation of progenitor cells successfully enhanced vascular development by in situ differentiation and proliferation within ischemic organs.[8] On the basis of these findings, the beneficial property of endothelial progenitor cells is attractive for angiogenic cellular interventions and as cell-mediated vehicles for gene therapy applications targeting regeneration of ischemic tissue and of failing hearts.

Stem Cell Differentiation to Muscle Cells

The principal aim is to transplant cells of primarily noncardiac origin, such as human bone marrow-derived mononuclear cells containing human stem cells. These cells may operate as a precursor of heart muscle tissue and of coronary blood vessel cells. Human bone marrow contains hematopoietic (1% to 2%) and mesenchymal stem cells (<0.05%). Both types of stem cells may contribute to heart muscle repair. Hematopoietic stem cells are progenitor cells for many types of cells, eg, endothelial cells, which may also differentiate to heart muscle cells. Mesenchymal stem cells are progenitor cells for types of cells such as heart muscle cells, as well as for a variety of cells of noncardiac concern. Recent results in mouse experiments suggest the potency of extracardiac progenitor cells for transdifferentiation into new cardiomyocytes after acute experimental myocardial infarction.[4] Bone marrow cells cultured with 5-azacytidine differentiated into cardiac-like muscle cells in culture and in vivo in ventricular scar tissue in pigs and improved myocardial function.[9] In clinical myocardial infarction, evidence has been provided that autologous bone marrow stem cells may regenerate in infarcted myocardium and improve myocardial perfusion of the infarct zone.[3] Studies with transplanted human hearts have shown that adult humans have extracardiac progenitor cells capable of migrating to and repopulating damaged myocardium, a process occurring at very low levels.[10] Recently, cases have been described in which a male patient receives a heart from a female donor, which provided an opportunity to test whether progenitor cells translocate from the recipient to the graft on the basis of Y chromosome labeling.[11] Results showed that myocytes, coronary arterioles, and capillaries that had a Y chromosome made up 7% to 10% of those in the donor hearts and were proliferative. This indicates a regenerative capacity of the transplanted myocardium. Thus, there is growing evidence for a repair function of extracardiac cells, eg, from bone marrow in the case of cardiac lesion and the necessity of myocardial healing, although these results are not unanimously approved.[12]

Milieu-Dependent Differentiation and Enhanced Environment

Studies from several species demonstrate that bone marrow-derived stem cells are stem cells for various mesenchymal tissues. The cells are therefore not simply stromal precursors, but precursors of peripheral tissues, such as heart muscle.[13] Normal growth and ultimate stem cell fate depend on engraft-



Delivery options for stem cell transfer modalities to the heart. The red colored area represents apical lesion of the left ventricle by myocardial infarction. The balloon catheter is localized in the infarct-related artery and is placed above the border zone of the infarction. Blue and green arrows suggest the possible route of cell infusion and migration into the infarct. The 2 small figures depict the transendocardial and intramyocardial route of administration. RCA indicates right coronary artery; LAD, left anterior descending coronary artery; and CFX, circumflex artery.

ment in an appropriate "niche." Nonetheless, the mechanisms by which the local milieu influences stem cell differentiation are as yet undetermined. Thus, it seems that the fate of bone marrow stem cells is determined by the environment in which they engraft rather than by an intrinsically programmed fate. Therefore, enhancement of functional activity of the specific organ's niche for heart muscle, eg, by positive inotropic (pharmacologic augmentation of contractility) or by positive chronotropic stimuli (heart rate increase by exercise), may promote and intensify the transdifferentiation of bone marrow-derived stem cells to the cardiomyocyte phenotype. After an injury, eg, myocardial infarction, or a cellular damage, eg, in severe pressure or volume overload of the heart, specific factors, including cytokines, stem cell factor, and various growth factors, that stimulate cell replication and substitution in the injured tissue are released by the surrounding cells. In addition, transplanted stem cells, differentiating to cardiomyocytes, become indistinguishable over time from the surrounding cardiomyocytes, and they begin to express the contractile proteins specific for striated heart muscle, including desmin, a-myosin, heavy chain, a-actinin, and phospholamban at levels that are the same as in the host cardiomyocytes.[14] This transdifferentiation process is more pronounced in injured tissue than in healthy organs and may be intensified when the heart as the recipient organ contributes to its enhanced environment by high chronotropic and inotropic activity. Thus, regionally large concentrations of stem cells and increased mechanical activity of the recipient heart muscle may provide a favorable environment for successful engraftment of stem cells after cardiac injury.

Route of Cell Administration

The appropriate route of cell administration to the damaged organ is an essential prerequisite for the success of organ repair (Figure). High cell concentrations within the area of interest and prevention of homing of transplanted cells into other organs are desirable. Therefore, targeted and regional administration and transplantation of cells should be preferred. Below, several special routes of administration are described.

- In regional heart muscle disease, as in myocardial infarction, selective cell delivery by intracoronary catheterization techniques leads to an effective accumulation and concentration of cells within the infarcted zone. This can be realized in humans with bone marrow-derived cells.[15] With intracoronary administration, all cells must pass the infarct and peri-infarct tissue during the immediate first passage. Accordingly, with the intracoronary procedure, the infarct tissue can be enriched with the maximum available number of cells at all times. Further developments of catheterization systems for various clinical studies are needed.
- The transendocardial and transpericardial route of application has been used in large animal experiments[16] and was also recently tested in patients.[17] The main potential advantage of the surgical procedure is injection under visualization, which allows anatomic identification of the target area and even distribution of the injections. The safety and feasibility of catheter-based transendocardial injection was demonstrated in large animal studies,[18] and initial clinical experience in 19 patients using intramyocardial gene trans-

fer showed similar safety profiles.[19] Current clinical experience is limited to one injection system, using electromechanical mapping to generate 3-dimensional left ventricular reconstruction before the injection. Intraventricular catheter manipulation, however, can injure the myocardium, inducing ventricular premature beats and short runs of ventricular tachycardia. In certain cases, this precludes injection to the more arrhythmogenic zones, and it may extend the duration of the procedure and should always be carefully monitored. Each injection catheter is tested for cell biocompatibility to assure no mechanical or functional damage to cells being propelled under pressure through the narrow injection needle. Future developments with steerable transendocardial injection and delivery systems with mapping of the injured zone are needed. Transendocardial injection of autologous bone marrow cells has also been performed as part of several pilot and phase I studies. Safety and feasibility data are still pending and efficacy parameters need large randomized clinical trials.

- The intravenous route of administration is easiest. The main disadvantage, however, is that approximately only 3% of normal cardiac output will flow per minute through the left ventricle, and it is also limited because of transpulmonary first-pass attenuation effect on the cells. Therefore, this administration technique will require many circulation passages to enable infused cells to come into contact with the infarct-related artery. During that time, homing of infused cells to other organs will considerably reduce the number of cells that will populate the infarcted area.
- Some major cell types, such as skeletal myoblasts, have the disadvantage of an emboligenic potency when delivered systemically. Therefore, intramyocardial injection during open-heart surgery has been tested. This procedure has also been used in humans.[20] However, the therapeutic effect is limited because of severe arrhythmogenic complications. Another approach implanted autologous bone marrow cells during open-heart surgery and could show improvement in myocardial perfusion in 3 of 5 treated patients.[21]

Detection of Transplanted Stem Cells

An important clinical problem will be the identification and localization of transplanted autologous stem cells within the injured area of the heart. The transplanted cell or cell population is a single unit in a complex biological network of other cells. Therefore, for both localization and fate mapping of stem cells within the target organ, specific cell markers are desirable. Thus, analysis of stem cell behavior will presume (1) in situ labeling of a single cell or a transplanted cell population or (2) transplantation of already in vitro labeled cells or cell populations. For labeling in animal experiments, retroviral transduction with a marker gene or labeling with thymidine or bromodeoxyuridine (BrdU) have been used. For clinical detection of stem cells, magnetic labeling and in vivo tracking of bone marrow cells by the use of magnetodendrimers or radioactive detection methods may be useful. Myocardial biopsies in humans hardly will be justifiable under these circumstances. Thus, localization and fate mapping of stem cells in the region of myocardial injury will represent an important task for experimental and clinical stem cell research in the future, as well as for the assessment of time course of proliferation in the recipient new cell homes and for the evaluation of proper cell function after full transdifferentiation. First results through the detection of the reporter gene *LacZ*, by identification of β-galactosidase–positive cells in tissue section and chromosome analysis by fluorescence in situ hybridization (FISH) techniques are encouraging.[22]

Stem Cells for Cardiac Wound Repair: A Joint Clinical and Experimental Approach

In the regenerating tissues, stem cells and progenitor cells in the microenvironment both take part in the renewal process. Bone marrow cells injected or mobilized to the damaged myocardium were shown to behave as cardiac stem cells with remarkable plasticity, giving rise to myocytes, endothelial cells, and smooth muscle cells.[23] In the case of human infarcted tissue, autologous bone marrow cells have shown to be highly effective in wound repair in terms of regenerating heart muscle and improving perfusion in the infarcted and border zone area.[24,25] Clinical studies therefore are necessary — in parallel to basic and experimental investigations — analyzing the promising prerequisites for clinical wound repair, preferably the optimum cell administration to the region of interest of the heart, eg, the infarcted tissue, and their optimum concentration and accumulation by different catheter-based techniques.

Moreover, catheter-guided cell transfer to the human heart has the unique advantages of being safe under local anesthesia and during routine cardiac catheterization, being fast, taking between 20 to 40 minutes for the whole procedure, and allowing the administration of bone marrow cells in abundance, selected or non-selected, from bone marrow puncture to the region of interest, which permits a much greater availability of stem cells for the heart than the normal wound healing in various heart diseases or in cardiac transplantation models per se would bring about.[15]

Experimental studies will be needed simultaneously to differentiate between the therapeutically most successful kinds of bone marrow cells:

Global bone marrow containing all mononuclear bone marrow cells or specifically selected subfractions, as isolated cell fractions containing preferably CD34+ or CD34-, CD45-, or AC133+ cells.

Analysis of the transdifferentiation of bone marrow cells to muscle cells and their contribution to the remodeling process in various heart diseases, including cardiac transplantation models.

Cardiac lesions may be multifactorial and include myocardial infarction, myocarditis, cardiomyopathy or cardiac remodeling due to severe pressure, and volume overload. It is

uncertain whether the same therapeutic approach and the same type of cells will be suitable for all of these different diseases. However, organ repair by stem cells represents a general biological mechanism. Thus, it will be one of the future tasks to find the most practical and specific way of evolving and targeting the healing potency of stem cells for selected cardiovascular diseases.

Therapeutic Alternatives in Advanced Heart Failure

Except for pharmacotherapeutics and other measures, the therapy of severe global heart failure and of advanced regional contraction insufficiency is based on nonpharmacological interventions. These are aimed at unloading the heart (cardiac assist device), harmonizing the electrical and mechanical course of contraction and relaxation (ventricular synchronization), restoring ventricular geometry by ventricular size diminution (myocardial left ventricular resection), or abolishing detrimental volume overload in mitral incompetence (repair of the mitral valve).[26,27] The clinical limitations of all of these approaches, which are aimed at reducing systolic wall stress and myocardial oxygen consumption,[28] justify the search for alternative therapeutic options that may beneficially modify the natural course of the disease. By stem cell-derived de novo restoration of damaged cells, replacement of destroyed and scarred tissue with the consecutive improvement of ventricular performance may be possible. It may be speculated that future therapeutical options of combined therapeutical strategies, eg, ventricular resynchronization together with myocardial stem cell repair, may result in additive therapeutical benefit.

Conclusions and Open Questions

Stem cell therapy represents a fascinating new approach for the management of heart diseases. Recent clinical results have shown the feasibility of adult autologous cell therapy in acute myocardial infarction in humans. However, many unresolved questions about experimental and clinical cardiology are still open for future research, especially many basic problems concerning, among others, the following issues:

- The long-term fate of transplanted stem cells in the recipient tissue.
- The ability of transplanted stem cells to find their optimum myocardial "niche."
- The potency of stem cells to transdifferentiate into heart muscle cells.
- The optimal angiogenic milieu needed for transplanted cells in hypoperfused tissue.
- The capability of the recipient tissue to enable an enhanced environment to offer optimum, milieu-dependent differentiation of engrafted cells.
- Specific detection of engrafted cells or cell populations by labeling techniques.
- The optimal time course of availability and application for stem cell replacement therapy in cardiovascular disease.
- The arrhythmogenic potential of implanted cells.
- The specific characterization of the progenitor cells that should be measured to predict therapeutic effect of transplanted cells.
- Development of safe and reproducible catheter-based delivery systems for depositing stem cells to recipient heart muscle.

Additional research is needed o explore the therapeutic merits of cell transplantation techniques while accepting the likelihood that possible adverse side effects may occur. With regard to the clinical practicability, ethical problems, and hazards of immunogenity, actual and future research will focus preferably on adult stem cells, whereas research on embryonic stem cells may emerge presumably into comparable clinical relevance in several years to come.

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EXHIBIT 2

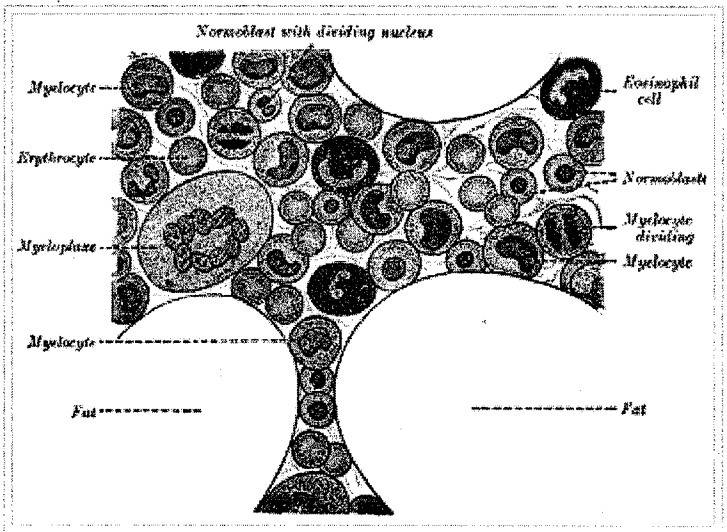
Bone marrow

From Wikipedia, the free encyclopedia

For the Dir en Grey album, see The Marrow of a Bone

Bone marrow (or ***medulla ossea***) is the soft tissue found in the hollow interior of bones. It is the place where most new blood cells are produced.

Contents



Gray's Anatomy illustration of cells in bone marrow.

Red and yellow marrow

There are two types of bone marrow: **red marrow** (also known as **myeloid tissue**) and **yellow marrow**. Red blood cells, platelets and most white blood cells arise in red marrow; some white blood cells develop in yellow marrow. The color of yellow marrow is due to the much higher number of fat cells. Both types of bone marrow contain numerous blood vessels and capillaries.

At birth, all bone marrow is red. With age, more and more of it is converted to the yellow type. Adults have on average about 2.6 kg of bone marrow, with about half of it being red. Red marrow is found mainly in the flat bones such as hip bone, breast bone, skull, ribs, vertebrae and shoulder blades, and in the cancellous ("spongy") material at the proximal ends of the long bones femur and humerus. Yellow marrow is found in the hollow interior of the middle portion of long bones.

In cases of severe blood loss, the body can convert yellow marrow back to red marrow in order to increase blood cell production.

Types of stem cells

Bone marrow contains two types of stem cells:

- Hematopoietic stem cells give rise to the three classes of blood cell that are found in the circulation: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes).

- Mesenchymal stem cells are found arrayed around the central sinus in the bone marrow. They have the capability to differentiate into osteoblasts, chondrocytes, myocytes, and many other types of cell. They also function as "gatekeeper" cells of the bone marrow.

Diseases involving the bone marrow

The normal bone marrow architecture can be displaced by malignancies or infections such as tuberculosis, leading to a decrease in the production of blood cells. In addition, cancers of the hematologic progenitor cells in the bone marrow can arise; these are the leukemias.

To diagnose diseases involving the bone marrow, a bone marrow biopsy is sometimes performed. This typically involves using a hollow needle to acquire a sample of red bone marrow from the crest of the ilium under local anesthesia.

Exposure to radiation or chemotherapy will kill many of the rapidly dividing cells of the bone marrow and will therefore result in a depressed immune system. Many of the symptoms of radiation sickness are due to damage to the bone marrow cells.

Donation and transplantation of bone marrow

It is possible to take hematopoietic stem cells from one person and then infuse them into another person, or into the same person at a later time. If donor and recipient are compatible, these infused cells will then travel to the bone marrow and initiate blood cell production. The stem cells can either be harvested directly from the red marrow in the crest of the ilium, usually under general anesthesia because the procedure involves numerous puncturings, or by administering certain drugs that stimulate the release of stem cells from the bone marrow into circulating blood and then filtering the stem cells out of that blood.

Bone marrow harvest

Transplantation from one person to another is performed in severe cases of disease of the bone marrow: the patient's marrow is first killed off with drugs or radiation, and then the new stem cells are introduced.

Before radiation therapy or chemotherapy in cases of cancer, some of the patient's hematopoietic stem cells are sometimes harvested, and later infused back when the therapy is finished to restore the immune system.

Bone marrow as a food

Though once used in various preparations, including pemmican, bone marrow for human consumption in America has recently fallen out of favor as a food. Now, it is commonly used only as a flavoring for soups and sauces, although dishes with intact bone marrow can still be found in some European

restaurants. Bone marrow is a source of protein and high in monounsaturated fats. These fats are known to decrease LDL cholesterol levels resulting in a reduced risk of cardiovascular disease, prompting some to make bone marrow a dietary staple. The actual health effects of the addition of bone marrow to the diet remain unknown.

See also

- Leukemia
- Bone marrow transplant
- Bone marrow biopsy
- Aplastic anemia
- Scotch Broth
- Osso buco

External links

- Bioweb at UWLAX *Microscopic slides of bone marrow, with explanations (http://bioweb.uwlax.edu/APlab/Table_of_Contents/Lab_11/Bone_Marrow_1/bone_marrow_1.htn*
- Marrow Balls (http://fooddownunder.com/cgi-bin/recipe.cgi?r=159368)
- Tostadas de tuetano (http://fooddownunder.com/cgi-bin/recipe.cgi?r=264236)

Lymphatic system [hide]
Bone marrow | Thymus (Hassall's corpuscles) | Spleen (White pulp, Periarteriolar lymphoid sheaths, Marginal zone, Red pulp) | Tonsils (Palatine, Lingual, Adenoid)
Mucosa-associated lymphoid tissue: Gut-associated lymphoid tissue | Peyer's patches
Lymph nodes: Subcapsular sinus | Paracortex | Lymph vessels
Lymph | Lymphocytes | High endothelial venules | Immune system

Bone and cartilage [hide]
cartilage: chondroblast, chondrocyte, perichondrium, *types* (hyaline, elastic, fibrous), fibrocartilage callus, metaphysis
bone: *ossification* (intramembranous, endochondral, epiphyseal plate), *cycle* (osteoblast, osteoid, osteocyte, osteoclast), *types* (cancellous, cortical), *regions* (epiphysis, diaphysis), *structure* (osteon/Haversian system, Haversian canals, endosteum, periosteum, Sharpey's fibres, lacunae, canaliculi, trabeculae, medullary cavity, **bone marrow**), *shapes* (long, short, flat, irregular, sesamoid)

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Category: Lymphatic system